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INHIBITION OF FLOWERING BY HEXAHYDROFLUORENE-9-CARBOXYLIC ACIDS RELATED TO ALLOGIBBERIC ACID

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Key Word Index—*Lemna perpusilla*; Lemnaceae; 1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acids; flowering inhibitors, allogibberic acid analogues.

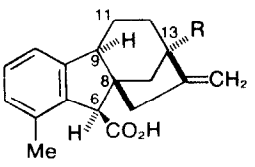
Abstract—1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acid isomers have been prepared by reduction of fluorene-9-carboxylic acid. Hexahydrofluorene-9-carboxylic acid **(5)** produce inhibition of flowering and vegetative frond development in *Lemna perpusilla* 6746 similar to that observed with allogibberic acid. The stereochemical requirements for this type of biological activity in allogibberic acids and hexahydrofluorene-9-carboxylic acids are considered.

INTRODUCTION

ALLOGIBBERIC acid **(1)** has recently been shown[1] to be the aqueous decomposition[2,3] product of gibberellic acid (GA_3) **(3)** responsible for inhibition of flowering and associated vegetative growth effects in the duckweed *Lemna perpusilla* Torr., strain 6746.[4,5] 13-Deoxyallogibberic acid **(2)**, derived from gibberellin A_7 (GA_7) **(4)** was prepared and found to be a more active flowering inhibitor than allogibberic acid in *L. perpusilla* assays.[1] To examine whether a further simplification of the molecule might still yield active compounds, the hexahydrofluorene-9-carboxylic acids **(5)** and **(6)** have now been synthesized. Compound **(5)** is shown to be an equally effective flowering inhibitor as allogibberic acid in *L. perpusilla* assays.[6] Their activity in these assays is compared with the much weaker activity of the fully aromatic "morphactins"[7] **(7)** and **(8)**.

The stereochemical requirements for flowering inhibition and associated vegetative growth effects in *L. perpusilla* by allogibberic acids, which were indicated in the earlier study,[1] have also been investigated. 6-*epi* and 9-*epi*-Allogibberic acids **(9)** and **(10)** have been investigated and both show little effect on flowering or vegetative growth of *L. perpusilla*.

[1] PRYCE, R. J. (1973) *Phytochemistry* **12**, 1745.
[2] PRYCE, R. J. (1973) *Phytochemistry* **12**, 507.
[3] PRYCE, R. J. (1974) *J. C. S. Perkin I* 1179.
[4] HODSON, H. K. and HAMNER, K. C. (1971) *Plant Physiol.* **47**, 726.
[5] HILLMAN, W. S. (1969) in *The Induction of Flowering—Some Case Histories* (EVANS, L. T. ed.), p. 186, MacMillan, Melbourne.
[6] U.K. Patent Application No. 1848/74.
[7] SCHNEIDER, G. (1970) *Ann. Rev. Plant Physiol.* **21**, 499.



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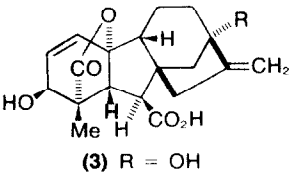

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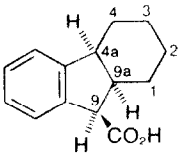

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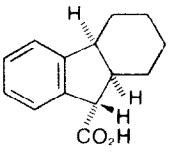

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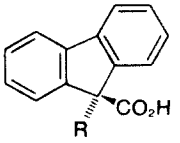

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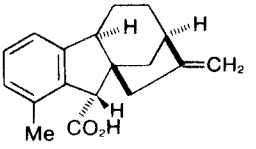

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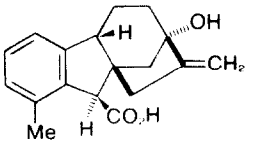

(10)

RESULTS AND DISCUSSION

Two routes were successfully applied to the synthesis of 1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acid system. Direct hydrogenation of fluorene-9-carboxylic acid (**7**) over Adams catalyst at room temperature and medium pressure yielded, *inter alia*, a mixture of hexahydrofluorene-9-carboxylic acid isomers as shown by GC–MS and NMR analysis of the total reduction product. One hexahydro-isomer predominated (*ca* 60% of the total hexahydro isomers) and this was isolated, characterized and assigned structure (**5**), see below. The second synthetic route was lithium-liquid ammonia reduction of fluorene-9-carboxylic acid followed by hydrogenation of the first crude partial reduction product at room temperature and pressure. This procedure gave, *inter alia*, a similar mixture of hexahydrofluorene-9-carboxylic acid isomers to the first route above. Again one isomer predominated and this, after isolation and characterization was found to be identical with the product (**5**) above. The isolated yield of (**5**) from both synthetic routes was *ca* 30% from (**7**). In both syntheses side products consisted of dodecahydro-, and some tetrahydro- and decahydrofluorene-9-carboxylic acids together with some starting material (**7**) as indicated by GC–MS determination. Some decarboxylation appears to accompany the direct catalytic reduction of (**7**). The two-stage procedure has the advantage of producing a more easily isolable product.

The methyl ester of the hexahydrofluorene-9-carboxylic acid (**5**) was epimerized to its 9-epimer (**6**) ("9-*epi*-hexahydrofluorene-9-carboxylic acid") 87%, containing 13% of (**5**) by NMR analysis. Coupling constants of the 9,9a protons in the NMR spectra of (**5**) and (**6**) (5·8 and 6·2 Hz respectively) were of little value in the assignment of the relative 9.9a stereochemistries of (**5**) and (**6**). MacMillan *et al.*[8] had previously found vicinal proton–proton coupling constants J_{cis} 7·0–7·3 and J_{trans} 8·0–9·5 Hz in the cyclopentane ring of some phenylindane derivatives. GLC analysis of the epimerization product of (**5**) and of (**5**) itself was hindered by thermal isomerization of the methyl esters of (**5**) and (**6**) during GLC. This was demonstrated by a GC–MS study where the methyl ester of (**5**) was chromatographed by injection of a solution of it in MeOD onto the GLC which had been pretreated by injection of MeOD. The GLC peaks corresponding to methyl esters of (**5**) and of (**6**) which

[8] MACMILLAN, J., MARTIN, I. L. and MORRIS, D. J. (1969) *Tetrahedron* **25**, 905.

were produced were examined by MS and both showed partial incorporations of one deuterium atom into both methyl esters as judged by their parent ions.

The assignment of the stereochemistries **(5)** and **(6)** to the synthesized hexahydrofluorene-9-carboxylic acid above and its 9-epimer respectively is based on the following observations. Hydrogen addition at C-9a would be expected to be *anti* to the carboxyl group[9] and this is confirmed by the fact that the less stable epimer **(5)** is produced. The 4a-H stereochemistry in **(5)** and **(6)** is supported by MS evidence which has been recorded in detail elsewhere.[10] Loss of a methyl formate (HCO_2Me) fragment from parent ions in MSs of the epimeric 9- and 6-allogibberic acid methyl esters [methyl esters of **(1)**, **(9)** and **(10)**] has been shown to involve the 9-hydrogen and to be dependent on the relative stereochemistries of C-6 and C-9.[10] Loss of methyl formate is preferred when the 9-hydrogen and 6-carbomethoxyl are *cis* as in **(9)** and **(10)**, and loss of a carbomethoxyl radical is preferred when these groups are *trans* as in **(1)**. It was found that the methyl ester of the hexahydrofluorene-9-carboxylic acid, prepared by the two syntheses above and assigned structure **(5)**, preferentially lost a carbomethoxyl radical from its parent ion in its MS and that the methyl ester of its 9-epimer, assigned structure **(6)**, showed a preferential loss of methyl formate. These preferred fragmentations are consistent with *trans*-9-carboxyl,4a-H in **(5)** and *cis*-9 carboxyl,4a-H relative stereochemistries in **(6)**.

The synthetic hexahydrofluorene-9-carboxylic acids were tested as inhibitors of flowering and vegetative frond growth in bioassays with *L. perpusilla* and the results are shown in Table 1. The isomer **(5)** is seen to be at least an equally effective flowering inhibitor as allogibberic acid in this assay. Somewhat lower activity is observed with the sample of the 9-epimeric hexahydrofluorene-9-carboxylic acid **(6)**. The unseparated mixture of hexahydrofluorene-9-carboxylic acid isomers produced by one of the synthetic routes above which contained mainly **(5)** and smaller amounts of the other three possible isomers including **(6)**, see above, is no more inhibitory to flowering than pure **(5)** (experiment 8, Table 1). The hexahydrofluorene-9-carboxylic acid isomer prepared here **(5)** is considerably more active in *L. perpusilla* assay than its fully aromatic counterpart and precursor, fluorene-9-carboxylic acid **(7)**. 9-Hydroxyfluorene-9-carboxylic acid **(8)** is generally considered to be a more active "morphactin" than **(7)**[7] both as a flowering inhibitor and inhibitor of frond development in *L. perpusilla* assays (Table 1) it is almost inactive except at the highest concentrations used, where its morphogenetic effects become visible.

The results above clearly demonstrate that a basic structural unit of allogibberic acid **(1)**, that of 1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acid, can produce similar biological effects in *L. perpusilla*. Present results do not permit any firm conclusions regarding the stereochemical requirements of the 9-substituted hexahydrofluorene system for flowering inhibitory activity and inhibition of vegetative frond development.

In the allogibberic acid system itself, stereochemical requirements for activity in *L. perpusilla* assays are clearer. Earlier results[1] had shown that 9-*epi*-allogibberic acid **(10)** was relatively inactive compared with allogibberic acid. This stereochemical point has now been investigated further (see Table) using a wider range of concentrations of 9-*epi*-allogibberic acid and also with the carboxyl epimer, 6-*epi*-allogibberic acid **(9)**. Both these 6- and 9-epimers of allogibberic acid are much less active as flowering inhibitors than allogibberic acid and they are also only very weak inhibitors of frond development. The only stereochemistry-activity relationship which may exist in both the allogibberic acids and the

[9] HOUSE, H. O. (1965) *Modern Synthetic Reactions*, p. 16, Benjamin, New York.
[10] GRAY, R. T. and PRYCE, R. J. *J. C. S. Perkin II* In press.

1,2,3,4,4a,9a-hexahydrofluorene-9-carboxylic acids is a requirement for a *trans*-relationship of the carboxyl group and the γ-hydrogen in the substituted cyclopentane ring. Allogibberic acid **(1)**, 13-deoxyallogibberic acid **(2)** and the hexahydrofluorene-9-carboxylic acid isomer **(5)** have this relative stereochemistry and of the compounds investigated here these appear to be the most active in the *L. perpusilla* assays.

TABLE 1. INHIBITION OF FLOWERING IN *Lemna perpusilla* 6746 BY HEXAHYDROFLUORENE-9-CARBOXYLIC ACIDS AND ALLOGIBBERIC ACIDS

Test substance	Concn in culture medium (μg/ml)	Flowering (% of control) Expt. no. 1	2	3	4	5	6	7[1]	8	Mean relative frond area (estimated by eye) [control 100]
Allogibberic acid **(1)**	10	22	0	0	31	12	23	25		25
	3·3	52	24	35	72					
	1	84	73	80	91	69	77	95		50
	0·33	99		95						
	0·1	101					84	96		100
13-Deoxyallogibberic acid **(2)**	10							0		10
	1							40		40
	0·1							88		100
Hexahydrofluorene-9-carboxylic **(5)**	10	0	3		43	4	0		0†	25
	3·3	10	3		61					
	1	52	42		79	73	46		72†	50
	0·33	89	66		97					
	0·1	96	95		97		104		100†	100
9-*epi*-Hexahydrofluorene-9-carboxylic acid **(6)***	10		0			10				25
	3·3		47							
	1		65			78				100
	0·33		85							
	0·1		96							100
"Morphactins" fluorene-9-carboxylic acid **(7)**	10	12				17	0		3	50
	3·3	77								
	1	97				99	103		92	100
	0·3	99								
	0·1	103					100		105	100
"Morphactins" 9-hydroxyfluorene 9-carboxylic acid **(8)**	10				75‡	58‡				100
	3·3				99					
	1				101	97				100
	0·3				96					
	0·1				104					100
9-*epi*-Allogibberic acid **(10)**	10			90		86				50
	3·3			99						
	1			101		99				100
	0·3			99						
	0·1			93						100
6-*epi*-Allogibberic acid **(9)**	10			90		74				50
	3·3			96						
	1			100		100				100
	0·3			101						
	0·1			99						100

* Contains *ca* 13% of **(5)** by NMR.

† Mixture of hexahydrofluorene isomers from Li/NH_3 reduction and hydrogenation used in expt. 8, **(5)** was the major component (*ca* 60%) the remainder consisted of approximately equal proportions of the other three possible isomers (GLC–MS analysis).

‡ Twisted roots and fronds produced at 10 μg/ml.

EXPERIMENTAL

Bioassays with *L. perpusilla* 6746 were carried out as previously described;[1] controls were 70–80% flowering. 6-*epi*-Allogibberic acid was prepared as in Reference 11 from allogibberic acid. Allogibberic acid and 9-*epi*-allogibberic acid were obtained as previously described.[2] 100 MHz NMR spectra were determined for $CDCl_3$ soln with $SiMe_4$ as internal standard with a Varian HA100 instrument. Methylations, GLC and GC–MS on 1% OV17 column were performed as previously;[1] with column temp. 150° and N_2 flow rate 45 ml/min the methyl ester of the hexahydrofluorene-9-carboxylic acid isomers **(5)** and **(6)** had R_t's of 6·1 and 5·5 min respectively, the other 2 isomers of **(5)** had R_t's of 4·9 and 5·3 min and under the same conditions the methyl esters of fluorene-9-carboxylic acid **(7)** had R_t 10·9 min. Petrol had b.p. 60–80.

Synthesis of 1,2,3,4,4*a*,9*a*-*hexahydrofluorene*-9-*carboxylic acid* **(5)**. (a) Fluorene-9-carboxylic acid (4 g, $1{\cdot}9 \times 10^{-2}$ mol) in glacial HOAc (250 ml) was hydrogenated over Adams catalyst (400 mg) in a Parr apparatus at 3 atm H_2 pressure and room temp. When three equivalents of H_2 had been taken up, which occurred in 1–6 days depending on the batch of catalyst used, the catalyst was removed by filtration through hard filter paper and evaporated *in vacuo*. An aliquot of the crude product was methylated and analysed by GLC–MS. The product was chromatographed on a column of silica gel and hexahydrofluorene-9-carboxylic acid **(5)** was eluted with 75% $CHCl_3$ in *n*-hexane to 100% to $CHCl_3$ (GLC monitoring). **(5)** Could be crystallized from some fractions from Me_2CO/petrol, however, crystallization mother liquors and the bulk of the fractions were subjected to preparative TLC on silica gel [solvent system EtOAc–petrol (1:1)]. Bands corresponding to **(5)** were extracted with EtOAc and the combined extracts were crystallized from Me_2CO/petrol to give **(5)**. The combined yield of crystalline **(5)** was 1·32 g or 32%. This crystalline product was identical with the fully characterized product **(5)** from (b) below by IR and NMR spectroscopy and by GLC–MS analysis. (b) Fluorene-9-carboxylic acid (10 g, $4{\cdot}76 \times 10^{-2}$ mol) was dissolved and stirred in refluxing liquid NH_3 (400 ml). Lithium (4 g, $5{\cdot}7 \times 10^{-1}$ mol) was then added during 0·5 hr then the reaction was allowed to continue refluxing and stirring for 1·75 hr. EtOH (200 ml) was then added during 0·5 hr after which NH_3 was evaporated in a stream of N_2. Residual EtOH was evaporated *in vacuo* at 30°. The product was dissolved in H_2O (300 ml), acidified with 4 N HCl (200 ml) and extracted with Et_2O (3 × 250 ml). The extract was then dried (Na_2SO_4) and evaporated *in vacuo*. The product was dissolved in EtOAc (350 ml) and hydrogenated at room temperature and pressure over Adams catalyst (1 g) which had been prehydrogenated in EtOAc (50 ml). Hydrogenation was continued for 48 hr until 1·45 equivalents of H_2 had been taken up. The reaction mixture was then filtered through a bed of celite and evaporated to dryness *in vacuo*. An aliquot of the final crude product was methylated and analysed by GLC–MS. Crystallization of the crude product (9·50 g) from Me_2CO/petrol gave pure hexahydrofluorene-9-carboxylic acid **(5)** (3·3 g, 32% yield) m.p. 176–185 (decomp.); (Found C, 77·6; H, 7·5. $C_{14}H_{16}O_2$ requires: C, 77·7; H, 7·5%); MS M^+ 216, GC–MS of Me ester M^+ 230; ν_{max} (Nujol) 3300–2300 (OH), 1713 and 1693 (CO_2H), 1602 (Ar, C=C), 762, 737, 682 cm^{-1}; λ_{max}^{EtOH} [nm (ϵ)] 273 (1070), 266 (970), 260 (620), *sh* 253 (350), *sh* 247 (190); NMR (τ) (assignments based on double resonance experiments) 8·0–9·2 *m* (3 × $-C\underline{H}_2-$), 7·70 *br d* ($-C\underline{H}_2-$), 7·14 *m* (9a-<u>H</u>), 6·76 *m* (4a-<u>H</u>), 5·94 *d J* 5·8 Hz (9-<u>H</u>), 2·77 *m* (3 × Ar <u>H</u>), 2·50 *m* (1 × Ar <u>H</u>), −0·09 *s* ($-CO_2\underline{H}$).

Synthesis of 9-epi-*hexahydrofluorene*-9-*carboxylic acid* **(6)**. Hexahydrofluorene-9-carboxylic acid **(5)** (400 mg) in MeOH (10 ml) was treated with excess ethereal CH_2N_2 at room temp. for 2 min. The reaction mixture was evaporated to dryness *in vacuo*. The ester was not isolated but was immediately dissolved in MeOH (3 ml) and 1·5 N NaOH (40 ml) was added. The solution was refluxed under N_2 for 2 hr then cooled and extracted firstly with EtOAc (3 × 25 ml) then acidified to pH 2 with 4 N HCl and extracted with EtOAc (3 × 25 ml). After drying (Na_2SO_4) the acid extract was evaporated to dryness *in vacuo* to give a gum (300 mg). The product was then molecularly distilled at 90–120°/0·2 mmHg to give 9-*epi*-hexahydrofluorene-9-carboxylic acid **(6)** as a viscous oil (290 mg, 72% yield) which contained 13% of **(5)** by NMR spectroscopy (see below); GC–MS of the Me ester M^+ 230; ν_{max} (liq. film) 3500–2200 (OH), 1704 (CO_2H), 1603 (Ar, C=C), 752, 736, 724, 687 cm^{-1}; λ_{max}^{EtOH} [nm (ϵ)] *sh* 274 (700), 272 (820), 266 (930), 260 (660), 5*h* 253 (430), *sh* 247 (310); NMR (τ) (assignments by analogy with **(5)** above, spectrum contained 13% of spectrum of **(5)** based on 9-<u>H</u> resonances—not recorded here) 8·0–9·0 *m* (4 × $-C\underline{H}_2-$), 7·18 *m* (9a-<u>H</u>), 6·70 *m* (4a-<u>H</u>), 6·24 *d J* 6·2 Hz (9-<u>H</u>), 2·79 *m* (3 × Ar <u>H</u>), 2·62 *m* (1 × Ar <u>H</u>), −1·00 *s* ($-CO_2\underline{H}$).

Acknowledgements—I thank Rosa Sanchez and Linda Linton for technical assistance with this work.

[11] GROVE, J. F. and MULHOLLAND, T. P. C. (1960) *J. Chem. Soc.* 3007.